

What is claimed is:

1. A composition comprising a cleavage structure, said cleavage structure comprising:
  - 5 a) a target nucleic acid having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region and said second region is located to and downstream from said third region;
  - 10 b) a first oligonucleotide having a 5' portion and a 3' portion, said 5' portion of said first oligonucleotide having a sequence complementary to said second region of said target nucleic acid and said 3' portion of said first oligonucleotide having a sequence complementary to said third region of said target nucleic acid; and
  - 15 c) a second oligonucleotide having a 5' portion, a central portion and a 3' portion, said 5' portion of said second oligonucleotide having a sequence complementary to said first region of said target nucleic acid, said central region of said second oligonucleotide having a sequence complementary to said second region of said target nucleic acid, and said 3' portion of said second oligonucleotide having a sequence that is not base-paired to said target nucleic acid and is selected from a set of oligonucleotides based on a
    - 20 following group of sequences,

1	4	6	6	1	3
2	4	5	5	2	3
1	8	1	2	3	4
1	7	1	9	8	4
1	1	9	2	6	9
1	2	4	3	9	6
9	8	9	8	10	9
9	1	2	3	8	10
8	8	7	4	3	1
1	1	1	1	1	2
2	1	3	3	2	2
3	1	2	2	3	2
4	1	4	4	4	2
1	2	3	3	1	1
1	3	2	2	1	4
3	3	3	3	3	4

4	3	1	1	4	4
3	4	1	1	3	3
3	6	6	6	3	5
6	6	1	1	6	5
7	6	7	7	7	5
8	7	5	5	8	8
2	1	7	7	1	1
2	3	2	3	1	3
2	6	5	6	1	6
4	8	1	1	3	8
5	3	1	1	6	3
5	6	8	8	6	6
8	3	6	5	7	3
1	2	3	1	4	6
1	5	7	5	4	3
2	1	6	7	3	6
2	6	1	3	3	1
2	7	6	8	3	1
3	4	3	1	2	5
3	5	6	1	2	7
3	6	1	7	2	7
4	6	3	5	1	7
5	4	6	3	8	6
6	8	2	3	7	1
7	1	7	8	6	3
7	3	4	1	6	8
4	7	7	1	2	4
3	6	5	2	6	3
1	4	1	4	6	1
3	3	1	4	8	1
8	3	3	5	3	8
1	3	6	6	3	7
7	3	8	6	4	7
3	1	3	7	8	6
10	9	5	5	10	10
7	10	10	10	7	9
9	9	7	7	10	9
9	3	10	3	10	3

9	6	3	4	10	6
10	4	10	3	9	4
3	9	3	10	4	9
9	10	5	9	4	8
3	9	4	9	10	7
3	5	9	4	10	8
4	10	5	4	9	3
5	3	3	9	8	10
6	8	6	9	7	10
4	6	10	9	6	4
4	9	8	10	8	3
7	7	9	10	5	3
8	8	9	3	9	10
8	10	2	9	5	9
9	6	2	2	7	10
9	7	5	3	10	6
10	3	6	8	9	2
10	9	3	2	7	3
8	9	10	3	6	2
3	2	5	10	8	9
8	2	3	10	2	9
6	3	9	8	2	10
3	7	3	9	9	10
9	10	1	1	9	4
10	1	9	1	4	1
7	1	10	9	8	1
9	1	10	1	10	6
9	6	9	1	3	10
3	10	8	8	9	1
3	8	1	9	10	3
9	10	1	3	6	9
1	9	1	10	3	1
1	4	9	6	8	10
3	3	9	6	1	10
5	3	1	6	9	10
6	1	8	10	9	6
5	9	9	4	10	3
2	10	9	1	9	5

10	10	7	2	1	9
10	9	9	1	8	2
1	8	6	8	9	10
1	9	1	3	8	10
9	6	9	10	1	2
1	10	8	9	9	2
1	9	6	7	2	9
4	3	9	3	5	1
5	11	10	14	12	1
7	12	4	13	3	2
5	5	4	4	12	9
2	13	13	11	13	13
10	2	5	4	12	7
11	7	4	11	6	4
12	12	1	9	11	11
12	9	4	14	12	6
12	7	13	2	9	11
9	11	3	4	1	3
10	5	12	11	4	4
4	13	7	12	1	5
9	13	10	11	11	6
10	14	14	10	1	3
2	14	1	10	4	5
10	12	12	7	11	10
9	11	2	12	8	11
2	8	5	2	12	14
1	8	13	3	7	8
9	4	7	5	4	2
13	2	12	7	1	12
11	10	9	7	5	11
8	12	2	2	12	7
5	2	14	3	4	13
1	8	8	1	5	9
14	5	11	10	13	3
14	1	4	13	2	4
4	4	5	11	3	10
10	9	2	3	3	11
11	4	8	14	3	4

5	1	14	8	11	2
14	3	11	6	12	5
13	4	4	1	10	1
6	10	11	6	5	1
5	8	12	5	1	7
4	5	9	6	9	2
13	2	4	4	2	3
11	2	2	5	9	3
8	1	10	12	2	8
12	7	9	11	4	1
12	1	4	14	3	13
11	2	7	10	4	1
3	4	12	11	11	11
3	3	4	2	12	11
1	5	9	4	2	1
6	1	12	2	10	5
10	5	1	12	2	14
2	11	7	9	4	11
7	4	4	5	14	12
12	5	2	1	10	12
5	9	2	11	6	1
12	14	3	6	1	14
5	9	11	10	1	4
2	5	12	14	10	10
4	5	8	4	5	6
10	12	4	6	12	5
4	2	1	13	6	8
9	10	10	14	5	3
6	14	10	11	3	3
2	9	10	12	5	7
13	3	7	10	5	12
6	4	1	2	5	13
6	1	13	4	14	13
2	12	1	14	1	9
4	11	13	2	6	10
1	10	7	4	5	8
7	2	2	10	13	4
8	2	11	4	6	14

4	8	2	6	2	3
7	1	12	11	2	9
5	6	10	4	13	4
5	10	4	11	9	3
3	11	9	3	2	3
8	15	6	20	17	19
21	10	15	3	7	11
11	7	17	20	14	9
16	6	17	13	21	21
10	15	22	6	17	21
15	7	17	10	22	22
3	20	8	15	20	16
17	21	10	16	6	22
6	21	14	14	14	16
7	17	3	20	10	7
16	19	14	17	7	21
20	16	7	15	22	10
20	10	18	11	22	18
18	7	19	15	7	22
21	18	7	21	16	3
14	13	7	22	17	13
19	7	8	12	10	17
15	3	21	14	9	7
19	6	15	7	14	14
4	17	10	15	20	19
21	6	18	4	20	16
2	19	8	17	6	13
12	12	6	17	4	20
16	21	12	10	19	16
14	14	15	2	7	21
8	16	21	6	22	16
14	17	22	14	17	20
10	21	7	15	21	18
16	13	20	18	21	12
15	7	4	22	14	13
7	19	14	8	15	4
4	5	3	20	7	16
22	18	6	18	13	20

19	6	16	3	13	3
18	6	22	7	20	18
10	17	11	21	8	13
7	10	17	19	10	14

wherein:

- (A) each of 1 to 22 is a 4mer selected from the group of 4mers consisting of WWWW, WWWX, WWY, WWXW, WWXX, WWXY, WWYW, WWYX, WWYY, WXWW, WXWX, WXWY, WXXW, WXXX, WXXY, WXYW, WXYX, WXY, WYWW, WYWX, WYWY, WYXW, WYXX, WYXY, WYYW, WYYX, WYYY, XWWW, XWWX, XWWY, XWXW, XWXX, XWXY, XWYW, XWYX, XWYY, XXWW, XXWX, XXWY, XXXW, XXXX, XXXY, XXYW, XXYX, XYY, XYWW, XYWX, XYWY, XYXW, XYXX, XYXY, XYYW, XYYX, XYYY, YWWW, YWWX, YWWY, YWXW, YWXX, YWXY, YWYW, YWYX, YWYY, YXWW, YXWX, YXWY, YXXW, YXXX, YXXY, YXYW, YXYX, YXYY, YYWW, YYWX, YYWY, YYXW, YYXX, YYYX, YYYW, YYYX, and YYYY, and
- (B) each of 1 to 22 is selected so as to be different from all of the others of 1 to 22;
- (C) each of W, X and Y is a base in which:
  - (i) (a) W = one of A, T/U, G, and C,  
X = one of A, T/U, G, and C,  
Y = one of A, T/U, G, and C,  
and each of W, X and Y is selected so as to be different from all of the others of W, X and Y,
  - (b) an unselected said base of (i) (a) can be substituted any number of times for any one of W, X and Y, or
  - (ii) (a) W = G or C,  
X = A or T/U,  
Y = A or T/U,  
and X ≠ Y, and
  - (b) a base not selected in (ii) (a) can be inserted into each sequence at one or more locations, the location of each insertion being the same in all the sequences;
- (D) up to three bases can be inserted at any location of any of the sequences or up to three bases can be deleted from any of the sequences;
- (E) all of the sequences of a said group of oligonucleotides are read 5' to 3' or are read 3' to 5'; and

wherein each oligonucleotide of a said set has a sequence of at least ten

contiguous bases of the sequence on which it is based, provided that:

(F) (I) the quotient of the sum of G and C divided by the sum of A, T/U, G and C for all combined sequences of the set is between about 0.1 and 0.40 and said quotient for each sequence of the set does not vary from the quotient for the combined sequences by more than 0.2; and

(II) for any phantom sequence generated from any pair of first and second sequences of the set  $L_1$  and  $L_2$  in length, respectively, by selection from the first and second sequences of identical bases in identical sequence with each other:

(i) any consecutive sequence of bases in the phantom sequence which is identical to a consecutive sequence of bases in each of the first and second sequences from which it is generated is less than  $((3/4 \times L) - 1)$  bases in length;

(ii) the phantom sequence, if greater than or equal to  $(5/6 \times L)$  in length, contains at least three insertions/deletions or mismatches when compared to the first and second sequences from which it is generated; and

(iii) the phantom sequence is not greater than or equal to  $(11/12 \times L)$  in length;

where  $L = L_1$ , or if  $L_1 \neq L_2$ , where  $L$  is the greater of  $L_1$  and  $L_2$ ; and

wherein any base present may be substituted by an analogue thereof.

2. The composition of claim 1, wherein one or more of said first and second oligonucleotides contain a 3'-terminal dideoxynucleotide.

5 3. The composition of claim 1, wherein the composition includes a plurality of said target nucleic acid sequences and a plurality of second oligonucleotide molecules such that each of said second oligonucleotide molecules has a distinct 3' region.

10 4. The composition of claim 3, wherein the composition includes at least ten said second oligonucleotide molecules or at least eleven said second oligonucleotide molecules, or at least twelve said second oligonucleotide molecules, or at least thirteen said second oligonucleotide molecules, or at least fourteen said second oligonucleotide molecules, or at least fifteen said second oligonucleotide molecules, or at least sixteen said second oligonucleotide molecules, or at least seventeen said second



oligonucleotide molecules, or at least eighteen said second oligonucleotide molecules, or at least nineteen said second oligonucleotide molecules, or at least twenty said second oligonucleotide molecules, or at least twenty-one said second oligonucleotide molecules, or at least twenty-two said second  
5 oligonucleotide molecules, or at least twenty-three said second oligonucleotide molecules, or at least twenty-four said second oligonucleotide molecules, or at least twenty-five said second oligonucleotide molecules, or at least twenty-six said second oligonucleotide molecules, or at least twenty-seven said second oligonucleotide molecules, or  
10 at least twenty-eight said second oligonucleotide molecules, or at least twenty-nine said second oligonucleotide molecules, or at least thirty said second oligonucleotide molecules, or at least thirty-one said second oligonucleotide molecules, or at least thirty-two said second oligonucleotide molecules, or at least thirty-three said second oligonucleotide molecules, or  
15 at least thirty-four said second oligonucleotide molecules, or at least thirty-five said second oligonucleotide molecules, or at least thirty-six said second oligonucleotide molecules, or at least thirty-seven said second oligonucleotide molecules, or at least thirty-eight said second oligonucleotide molecules, or at least thirty-nine said second  
20 oligonucleotide molecules, or at least forty said second oligonucleotide molecules, or at least forty-one said second oligonucleotide molecules, or at least forty-two said second oligonucleotide molecules, or at least forty-three said second oligonucleotide molecules, or at least forty-four said second oligonucleotide molecules, or at least forty-five said second  
25 oligonucleotide molecules, or at least forty-six said second oligonucleotide molecules, or at least forty-seven said second oligonucleotide molecules, or at least forty-eight said second oligonucleotide molecules, or at least forty-nine said second oligonucleotide molecules, or at least fifty said second oligonucleotide molecules, or at least sixty said second  
30 oligonucleotide molecules, or at least seventy said second oligonucleotide molecules, or at least eighty said second oligonucleotide molecules, or at least ninety said second oligonucleotide molecules, or at least one hundred said second oligonucleotide molecules, or at least one hundred and ten said second oligonucleotide molecules, or at least one hundred and twenty said  
35 second oligonucleotide molecules, or at least one hundred and thirty said second oligonucleotide molecules, or at least one hundred and forty said second oligonucleotide molecules, or at least one hundred and fifty said second oligonucleotide molecules, or at least one hundred and sixty said

second oligonucleotide molecules, or at least one hundred and seventy said second oligonucleotide molecules, or at least one hundred and eighty said second oligonucleotide molecules, or at least one hundred and ninety said second oligonucleotide molecules, or at least two hundred said second oligonucleotide molecules.

5. A method of detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products, the method comprising:
- a) providing:
    - i) a cleavage means,
    - 10 ii) a target nucleic acid, said target nucleic acid having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region, and said second region is located adjacent to and downstream from said third region;
    - 15 iii) a first oligonucleotide having a 5' and a 3' portion, said 5' portion of said first oligonucleotide having a sequence complementary to said second region of said target nucleic acid and said 3' portion of said first oligonucleotide having a sequence complementary to said third region of said target nucleic acid;
    - 20 iv) a second oligonucleotide having a 5' portion, a central portion and a 3' portion, said 5' portion of said second oligonucleotide having a sequence complementary to said first region of said target nucleic acid, said central portion of said second oligonucleotide having a sequence complimentary to said second region of said target nucleic acid, and said 3' portion of said second oligonucleotide having a sequence that is not base-
    - 25 paired to said target nucleic acid and is selected from a set of oligonucleotides based on a following group of sequences;

1	4	6	6	1	3
2	4	5	5	2	3
1	8	1	2	3	4
1	7	1	9	8	4
1	1	9	2	6	9
1	2	4	3	9	6
9	8	9	8	10	9
9	1	2	3	8	10
8	8	7	4	3	1
1	1	1	1	1	2
2	1	3	3	2	2

3	1	2	2	3	2
4	1	4	4	4	2
1	2	3	3	1	1
1	3	2	2	1	4
3	3	3	3	3	4
4	3	1	1	4	4
3	4	1	1	3	3
3	6	6	6	3	5
6	6	1	1	6	5
7	6	7	7	7	5
8	7	5	5	8	8
2	1	7	7	1	1
2	3	2	3	1	3
2	6	5	6	1	6
4	8	1	1	3	8
5	3	1	1	6	3
5	6	8	8	6	6
8	3	6	5	7	3
1	2	3	1	4	6
1	5	7	5	4	3
2	1	6	7	3	6
2	6	1	3	3	1
2	7	6	8	3	1
3	4	3	1	2	5
3	5	6	1	2	7
3	6	1	7	2	7
4	6	3	5	1	7
5	4	6	3	8	6
6	8	2	3	7	1
7	1	7	8	6	3
7	3	4	1	6	8
4	7	7	1	2	4
3	6	5	2	6	3
1	4	1	4	6	1
3	3	1	4	8	1
8	3	3	5	3	8
1	3	6	6	3	7
7	3	8	6	4	7

3	1	3	7	8	6
10	9	5	5	10	10
7	10	10	10	7	9
9	9	7	7	10	9
9	3	10	3	10	3
9	6	3	4	10	6
10	4	10	3	9	4
3	9	3	10	4	9
9	10	5	9	4	8
3	9	4	9	10	7
3	5	9	4	10	8
4	10	5	4	9	3
5	3	3	9	8	10
6	8	6	9	7	10
4	6	10	9	6	4
4	9	8	10	8	3
7	7	9	10	5	3
8	8	9	3	9	10
8	10	2	9	5	9
9	6	2	2	7	10
9	7	5	3	10	6
10	3	6	8	9	2
10	9	3	2	7	3
8	9	10	3	6	2
3	2	5	10	8	9
8	2	3	10	2	9
6	3	9	8	2	10
3	7	3	9	9	10
9	10	1	1	9	4
10	1	9	1	4	1
7	1	10	9	8	1
9	1	10	1	10	6
9	6	9	1	3	10
3	10	8	8	9	1
3	8	1	9	10	3
9	10	1	3	6	9
1	9	1	10	3	1
1	4	9	6	8	10

3	3	9	6	1	10
5	3	1	6	9	10
6	1	8	10	9	6
5	9	9	4	10	3
2	10	9	1	9	5
10	10	7	2	1	9
10	9	9	1	8	2
1	8	6	8	9	10
1	9	1	3	8	10
9	6	9	10	1	2
1	10	8	9	9	2
1	9	6	7	2	9
4	3	9	3	5	1
5	11	10	14	12	1
7	12	4	13	3	2
5	5	4	4	12	9
2	13	13	11	13	13
10	2	5	4	12	7
11	7	4	11	6	4
12	12	1	9	11	11
12	9	4	14	12	6
12	7	13	2	9	11
9	11	3	4	1	3
10	5	12	11	4	4
4	13	7	12	1	5
9	13	10	11	11	6
10	14	14	10	1	3
2	14	1	10	4	5
10	12	12	7	11	10
9	11	2	12	8	11
2	8	5	2	12	14
1	8	13	3	7	8
9	4	7	5	4	2
13	2	12	7	1	12
11	10	9	7	5	11
8	12	2	2	12	7
5	2	14	3	4	13
1	8	8	1	5	9

14	5	11	10	13	3
14	1	4	13	2	4
4	4	5	11	3	10
10	9	2	3	3	11
11	4	8	14	3	4
5	1	14	8	11	2
14	3	11	6	12	5
13	4	4	1	10	1
6	10	11	6	5	1
5	8	12	5	1	7
4	5	9	6	9	2
13	2	4	4	2	3
11	2	2	5	9	3
8	1	10	12	2	8
12	7	9	11	4	1
12	1	4	14	3	13
11	2	7	10	4	1
3	4	12	11	11	11
3	3	4	2	12	11
1	5	9	4	2	1
6	1	12	2	10	5
10	5	1	12	2	14
2	11	7	9	4	11
7	4	4	5	14	12
12	5	2	1	10	12
5	9	2	11	6	1
12	14	3	6	1	14
5	9	11	10	1	4
2	5	12	14	10	10
4	5	8	4	5	6
10	12	4	6	12	5
4	2	1	13	6	8
9	10	10	14	5	3
6	14	10	11	3	3
2	9	10	12	5	7
13	3	7	10	5	12
6	4	1	2	5	13
6	1	13	4	14	13

2	12	1	14	1	9
4	11	13	2	6	10
1	10	7	4	5	8
7	2	2	10	13	4
8	2	11	4	6	14
4	8	2	6	2	3
7	1	12	11	2	9
5	6	10	4	13	4
5	10	4	11	9	3
3	11	9	3	2	3
8	15	6	20	17	19
21	10	15	3	7	11
11	7	17	20	14	9
16	6	17	13	21	21
10	15	22	6	17	21
15	7	17	10	22	22
3	20	8	15	20	16
17	21	10	16	6	22
6	21	14	14	14	16
7	17	3	20	10	7
16	19	14	17	7	21
20	16	7	15	22	10
20	10	18	11	22	18
18	7	19	15	7	22
21	18	7	21	16	3
14	13	7	22	17	13
19	7	8	12	10	17
15	3	21	14	9	7
19	6	15	7	14	14
4	17	10	15	20	19
21	6	18	4	20	16
2	19	8	17	6	13
12	12	6	17	4	20
16	21	12	10	19	16
14	14	15	2	7	21
8	16	21	6	22	16
14	17	22	14	17	20
10	21	7	15	21	18

16	13	20	18	21	12
15	7	4	22	14	13
7	19	14	8	15	4
4	5	3	20	7	16
22	18	6	18	13	20
19	6	16	3	13	3
18	6	22	7	20	18
10	17	11	21	8	13
7	10	17	19	10	14

wherein:

- (A) each of 1 to 22 is a 4mer selected from the group of 4mers consisting of WWWW, WWWX, WWWY, WWWZ, WWXX, WWXY, WWYW, WWYX, WWYY, WXWW, WXWX, WXWY, WXXW, WXXX, WXXY, WXYW, WXYX, WXYZ, WYWW, WYWX, WYWY, WYXW, WYXX, WYXY, WYYW, WYYX, WYYY, XWWW, XWWX, XWWY, XWXW, XWXX, XWXY, XWYW, XWYX, XWYY, XXWW, XXWX, XXWY, XXXW, XXXX, XXXY, XXYW, XXYX, XXYY, XYWW, XYWX, XYWY, XYXW, XYXX, XYXY, XYYW, XYYX, XYYY, YWWW, YWWX, YWWY, YWXW, YWXX, YWXY, YWYW, YWYX, YWYY, YXWW, YXWX, YXWY, YXXW, YXXX, YXXY, YXYW, YXYX, YXYZ, YYWW, YYWX, YYWY, YYXW, YYXX, YYXY, YYYW, YYYX, and YYYY, and
- (B) each of 1 to 22 is selected so as to be different from all of the others of 1 to 22;
- (C) each of W, X and Y is a base in which:
  - (i) (a) W = one of A, T/U, G, and C,  
X = one of A, T/U, G, and C,  
Y = one of A, T/U, G, and C,  
and each of W, X and Y is selected so as to be different from all of the others of W, X and Y,
  - (b) an unselected said base of (i) (a) can be substituted any number of times for any one of W, X and Y, or
  - (ii) (a) W = G or C,  
X = A or T/U,  
Y = A or T/U,  
and X ≠ Y, and
  - (b) a base not selected in (ii) (a) can be inserted into each sequence at one or more locations, the location of each insertion being the same in all the sequences;
- (D) up to three bases can be inserted at any location of any of the



sequences or up to three bases can be deleted from any of the sequences;

- (E) all of the sequences of a said group of oligonucleotides are read 5' to 3' or are read 3' to 5'; and

wherein each oligonucleotide of a said set has a sequence of at least ten contiguous bases of the sequence on which it is based, provided that:

- (F) (I) the quotient of the sum of G and C divided by the sum of A, T/U, G and C for all combined sequences of the set is between about 0.1 and 0.40 and said quotient for each sequence of the set does not vary from the quotient for the combined sequences by more than 0.2; and
- (II) for any phantom sequence generated from any pair of first and second sequences of the set  $L_1$  and  $L_2$  in length, respectively, by selection from the first and second sequences of identical bases in identical sequence with each other:
- (i) any consecutive sequence of bases in the phantom sequence which is identical to a consecutive sequence of bases in each of the first and second sequences from which it is generated is less than  $((3/4 \times L) - 1)$  bases in length;
  - (ii) the phantom sequence, if greater than or equal to  $(5/6 \times L)$  in length, contains at least three insertions/deletions or mismatches when compared to the first and second sequences from which it is generated; and
  - (iii) the phantom sequence is not greater than or equal to  $(11/12 \times L)$  in length;

where  $L = L_1$ , or if  $L_1 \neq L_2$ , where  $L$  is the greater of  $L_1$  and  $L_2$ ; and

wherein any base present may be substituted by an analogue thereof;

- b) mixing said cleavage means, said target nucleic acid, said first and second oligonucleotides to create a reaction mixture under reaction conditions such that at least said 5' portion of said first oligonucleotide is annealed to said target nucleic acid and wherein at least said 5' and central portion of said second oligonucleotide is annealed to said target nucleic acid so as to create a cleavage structure and wherein the combined melting temperature of said complementary regions within said 5' and 3' portions of said first oligonucleotide when annealed to said target nucleic

acid is greater than the melting temperature of said 5' and central portion of said first oligonucleotide, and wherein cleavage of said cleavage structure occurs to generate non-target cleavage products; and

c) detecting said non-target cleavage products.

5        6.     The method of claim 5, wherein said reaction temperature is between approximately 50 and 70 degrees centigrade.

7.     The method of claim 5, wherein said target nucleic acid comprises single-stranded DNA.

10       8.     The method of claim 5, wherein said target nucleic acid comprises double-stranded DNA and prior to step (c), said reaction mixture is treated such that said double-stranded DNA is rendered substantially single-stranded.

9.     The method of claim 5, wherein said treatment to render said double-stranded DNA is rendered substantially single-stranded by increasing the temperature.

15       10.    The method of claim 5, wherein said target nucleic acid comprises RNA and wherein said first and second oligonucleotides comprise DNA.

11.    The method of claim 5, wherein said cleavage means comprises a thermostable 5' nuclease.

20       12.    The method of claim 11, wherein a portion of the amino acid sequence is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

13.    The method of claim 12, wherein said organism is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus* and *Thermus thermophilus*.

25       14.    The method of claim 5, wherein said source of target nucleic acid comprises a sample containing genomic DNA.

15.    The method of claim 5, wherein said reaction conditions comprise providing a source of divalent cations.

30       16.    The method of claim 15, wherein said divalent cation is selected from the group comprising  $Mn^{2+}$  and  $Mg^{2+}$  ions.

17.    The method of claim 5, wherein the method includes a plurality of said target nucleic acid sequences and a plurality of said second oligonucleotide molecules such that each of said second oligonucleotide molecules has a distinct 3' region.

35       18.    The method of claim 5, wherein the method includes at least ten said second oligonucleotide molecules or at least eleven said second oligonucleotide molecules, or at least twelve said second oligonucleotide molecules, or at least thirteen said second oligonucleotide molecules, or at

least fourteen said second oligonucleotide molecules, or at least fifteen said second oligonucleotide molecules, or at least sixteen said second oligonucleotide molecules, or at least seventeen said second oligonucleotide molecules, or at least eighteen said second oligonucleotide molecules, or at least nineteen said second oligonucleotide molecules, or at least twenty said second oligonucleotide molecules, or at least twenty-one said second oligonucleotide molecules, or at least twenty-two said second oligonucleotide molecules, or at least twenty-three said second oligonucleotide molecules, or at least twenty-four said second oligonucleotide molecules, or at least twenty-five said second oligonucleotide molecules, or at least twenty-six said second oligonucleotide molecules, or at least twenty-seven said second oligonucleotide molecules, or at least twenty-eight said second oligonucleotide molecules, or at least twenty-nine said second oligonucleotide molecules, or at least thirty said second oligonucleotide molecules, or at least thirty-one said second oligonucleotide molecules, or at least thirty-two said second oligonucleotide molecules, or at least thirty-three said second oligonucleotide molecules, or at least thirty-four said second oligonucleotide molecules, or at least thirty-five said second oligonucleotide molecules, or at least thirty-six said second oligonucleotide molecules, or at least thirty-seven said second oligonucleotide molecules, or at least thirty-eight said second oligonucleotide molecules, or at least thirty-nine said second oligonucleotide molecules, or at least forty said second oligonucleotide molecules, or at least forty-one said second oligonucleotide molecules, or at least forty-two said second oligonucleotide molecules, or at least forty-three said second oligonucleotide molecules, or at least forty-four said second oligonucleotide molecules, or at least forty-five said second oligonucleotide molecules, or at least forty-six said second oligonucleotide molecules, or at least forty-seven said second oligonucleotide molecules, or at least forty-eight said second oligonucleotide molecules, or at least forty-nine said second oligonucleotide molecules, or at least fifty said second oligonucleotide molecules, or at least sixty said second oligonucleotide molecules, or at least seventy said second oligonucleotide molecules, or at least eighty said second oligonucleotide molecules, or at least ninety said second oligonucleotide molecules, or at least one hundred said second oligonucleotide molecules, or at least one hundred and ten said second oligonucleotide molecules, or at least one hundred and twenty said second oligonucleotide molecules, or at least one hundred and thirty said second oligonucleotide molecules, or at

least one hundred and forty said second oligonucleotide molecules, or at least one hundred and fifty said second oligonucleotide molecules, or at least one hundred and sixty said second oligonucleotide molecules, or at least one hundred and seventy said second oligonucleotide molecules, or at  
 5 least one hundred and eighty said second oligonucleotide molecules, or at least one hundred and ninety said second oligonucleotide molecules, or at least two hundred said second oligonucleotide molecules.

19. The method of claim 5, wherein said 3' portion of said second oligonucleotide incorporates fluorescent molecule, a radiolabelled  
 10 nucleotide, digoxigenin, biotinylation and the like.

20. A method of analyzing a biological sample comprising a plurality of target nucleic acid molecules for the presence of a mutation or polymorphism at a locus of each target nucleic acid molecule, the method comprising:

15 a) providing:

i) a cleavage means,  
 ii) a plurality of target nucleic acid molecules, each of said target nucleic acid molecules having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from  
 20 said second region, and said second region is located adjacent to and downstream from said third region;

iii) a plurality of first oligonucleotide molecules, each having a 5' and a 3' portion, said 5' portion of said first oligonucleotide molecules having a sequence complementary to said second region of said target nucleic  
 25 acid molecules and said 3' portion of said first oligonucleotide molecules having a sequence complementary to said third region of said target nucleic acid molecules;

iv) a plurality of second oligonucleotide molecules, each having a 5' portion, a central portion and a 3' portion, said 5' portion of said second oligonucleotide molecules having a sequence complementary to said first  
 30 region of said target nucleic acid molecules, said central portion of said second oligonucleotide molecules having a sequence complimentary to said second region of said target nucleic acid molecules, and said 3' portion of said second oligonucleotide molecules having a sequence that is not base-  
 35 paired to said target nucleic acid and is selected from a set of oligonucleotides based on a following group of sequences,

2	4	5	5	2	3
1	8	1	2	3	4
1	7	1	9	8	4
1	1	9	2	6	9
1	2	4	3	9	6
9	8	9	8	10	9
9	1	2	3	8	10
8	8	7	4	3	1
1	1	1	1	1	2
2	1	3	3	2	2
3	1	2	2	3	2
4	1	4	4	4	2
1	2	3	3	1	1
1	3	2	2	1	4
3	3	3	3	3	4
4	3	1	1	4	4
3	4	1	1	3	3
3	6	6	6	3	5
6	6	1	1	6	5
7	6	7	7	7	5
8	7	5	5	8	8
2	1	7	7	1	1
2	3	2	3	1	3
2	6	5	6	1	6
4	8	1	1	3	8
5	3	1	1	6	3
5	6	8	8	6	6
8	3	6	5	7	3
1	2	3	1	4	6
1	5	7	5	4	3
2	1	6	7	3	6
2	6	1	3	3	1
2	7	6	8	3	1
3	4	3	1	2	5
3	5	6	1	2	7
3	6	1	7	2	7
4	6	3	5	1	7
5	4	6	3	8	6

6	8	2	3	7	1
7	1	7	8	6	3
7	3	4	1	6	8
4	7	7	1	2	4
3	6	5	2	6	3
1	4	1	4	6	1
3	3	1	4	8	1
8	3	3	5	3	8
1	3	6	6	3	7
7	3	8	6	4	7
3	1	3	7	8	6
10	9	5	5	10	10
7	10	10	10	7	9
9	9	7	7	10	9
9	3	10	3	10	3
9	6	3	4	10	6
10	4	10	3	9	4
3	9	3	10	4	9
9	10	5	9	4	8
3	9	4	9	10	7
3	5	9	4	10	8
4	10	5	4	9	3
5	3	3	9	8	10
6	8	6	9	7	10
4	6	10	9	6	4
4	9	8	10	8	3
7	7	9	10	5	3
8	8	9	3	9	10
8	10	2	9	5	9
9	6	2	2	7	10
9	7	5	3	10	6
10	3	6	8	9	2
10	9	3	2	7	3
8	9	10	3	6	2
3	2	5	10	8	9
8	2	3	10	2	9
6	3	9	8	2	10
3	7	3	9	9	10

9	10	1	1	9	4
10	1	9	1	4	1
7	1	10	9	8	1
9	1	10	1	10	6
9	6	9	1	3	10
3	10	8	8	9	1
3	8	1	9	10	3
9	10	1	3	6	9
1	9	1	10	3	1
1	4	9	6	8	10
3	3	9	6	1	10
5	3	1	6	9	10
6	1	8	10	9	6
5	9	9	4	10	3
2	10	9	1	9	5
10	10	7	2	1	9
10	9	9	1	8	2
1	8	6	8	9	10
1	9	1	3	8	10
9	6	9	10	1	2
1	10	8	9	9	2
1	9	6	7	2	9
4	3	9	3	5	1
5	11	10	14	12	1
7	12	4	13	3	2
5	5	4	4	12	9
2	13	13	11	13	13
10	2	5	4	12	7
11	7	4	11	6	4
12	12	1	9	11	11
12	9	4	14	12	6
12	7	13	2	9	11
9	11	3	4	1	3
10	5	12	11	4	4
4	13	7	12	1	5
9	13	10	11	11	6
10	14	14	10	1	3
2	14	1	10	4	5

10	12	12	7	11	10
9	11	2	12	8	11
2	8	5	2	12	14
1	8	13	3	7	8
9	4	7	5	4	2
13	2	12	7	1	12
11	10	9	7	5	11
8	12	2	2	12	7
5	2	14	3	4	13
1	8	8	1	5	9
14	5	11	10	13	3
14	1	4	13	2	4
4	4	5	11	3	10
10	9	2	3	3	11
11	4	8	14	3	4
5	1	14	8	11	2
14	3	11	6	12	5
13	4	4	1	10	1
6	10	11	6	5	1
5	8	12	5	1	7
4	5	9	6	9	2
13	2	4	4	2	3
11	2	2	5	9	3
8	1	10	12	2	8
12	7	9	11	4	1
12	1	4	14	3	13
11	2	7	10	4	1
3	4	12	11	11	11
3	3	4	2	12	11
1	5	9	4	2	1
6	1	12	2	10	5
10	5	1	12	2	14
2	11	7	9	4	11
7	4	4	5	14	12
12	5	2	1	10	12
5	9	2	11	6	1
12	14	3	6	1	14
5	9	11	10	1	4



2	5	12	14	10	10
4	5	8	4	5	6
10	12	4	6	12	5
4	2	1	13	6	8
9	10	10	14	5	3
6	14	10	11	3	3
2	9	10	12	5	7
13	3	7	10	5	12
6	4	1	2	5	13
6	1	13	4	14	13
2	12	1	14	1	9
4	11	13	2	6	10
1	10	7	4	5	8
7	2	2	10	13	4
8	2	11	4	6	14
4	8	2	6	2	3
7	1	12	11	2	9
5	6	10	4	13	4
5	10	4	11	9	3
3	11	9	3	2	3
8	15	6	20	17	19
21	10	15	3	7	11
11	7	17	20	14	9
16	6	17	13	21	21
10	15	22	6	17	21
15	7	17	10	22	22
3	20	8	15	20	16
17	21	10	16	6	22
6	21	14	14	14	16
7	17	3	20	10	7
16	19	14	17	7	21
20	16	7	15	22	10
20	10	18	11	22	18
18	7	19	15	7	22
21	18	7	21	16	3
14	13	7	22	17	13
19	7	8	12	10	17
15	3	21	14	9	7

19	6	15	7	14	14
4	17	10	15	20	19
21	6	18	4	20	16
2	19	8	17	6	13
12	12	6	17	4	20
16	21	12	10	19	16
14	14	15	2	7	21
8	16	21	6	22	16
14	17	22	14	17	20
10	21	7	15	21	18
16	13	20	18	21	12
15	7	4	22	14	13
7	19	14	8	15	4
4	5	3	20	7	16
22	18	6	18	13	20
19	6	16	3	13	3
18	6	22	7	20	18
10	17	11	21	8	13
7	10	17	19	10	14

wherein:

- (A) each of 1 to 22 is a 4mer selected from the group of 4mers consisting of WWWW, WWWX, WWWY, WWXW, WWXX, WWXY, WWYW, WWYX, WWYY, WXWW, WXWX, WXWY, WXXW, WXXX, WXXY, WXYW, WXYX, WXYX, WYWW, WYWX, WYWY, WYXW, WYXX, WYXY, WYYW, WYYX, WYYY, XWWW, XWWX, XWWY, XWXW, XWXX, XWXY, XWYW, XWYX, XWYY, XXWW, XXWX, XXWY, XXXW, XXXX, XXXY, XXYW, XXYX, XXYY, XYWW, XYWX, XYWY, XYXW, XYXX, XYXY, XYYW, XYYX, XYYY, YWWW, YWWX, YWWY, YWXW, YWXX, YWXY, YWYW, YWYX, YWYY, YXWW, YXWX, YXWY, YXXW, YXXX, YXXY, YXYW, YXYX, YXYY, YYWW, YYWX, YYWY, YYXW, YYXX, YYXY, YYYW, YYYX, and YYYY, and
- (B) each of 1 to 22 is selected so as to be different from all of the others of 1 to 22;
- (C) each of W, X and Y is a base in which:
- (i) (a) W = one of A, T/U, G, and C,
  - X = one of A, T/U, G, and C,
  - Y = one of A, T/U, G, and C,
- and each of W, X and Y is selected so as to be different from all of the others of W, X and Y,

- (b) an unselected said base of (i)(a) can be substituted any number of times for any one of W, X and Y, or
- (ii) (a) W = G or C,  
X = A or T/U,  
Y = A or T/U,  
and X ≠ Y, and
- (b) a base not selected in (ii)(a) can be inserted into each sequence at one or more locations, the location of each insertion being the same in all the sequences;
- (D) up to three bases can be inserted at any location of any of the sequences or up to three bases can be deleted from any of the sequences;
- (E) all of the sequences of a said group of oligonucleotides are read 5' to 3' or are read 3' to 5'; and

wherein each oligonucleotide of a said set has a sequence of at least ten contiguous bases of the sequence on which it is based, provided that:

- (F) (I) the quotient of the sum of G and C divided by the sum of A, T/U, G and C for all combined sequences of the set is between about 0.1 and 0.40 and said quotient for each sequence of the set does not vary from the quotient for the combined sequences by more than 0.2; and
- (II) for any phantom sequence generated from any pair of first and second sequences of the set  $L_1$  and  $L_2$  in length, respectively, by selection from the first and second sequences of identical bases in identical sequence with each other:
  - (i) any consecutive sequence of bases in the phantom sequence which is identical to a consecutive sequence of bases in each of the first and second sequences from which it is generated is less than  $((3/4 \times L) - 1)$  bases in length;
  - (ii) the phantom sequence, if greater than or equal to  $(5/6 \times L)$  in length, contains at least three insertions/deletions or mismatches when compared to the first and second sequences from which it is generated; and
  - (iii) the phantom sequence is not greater than or equal to  $(11/12 \times L)$  in length;

where  $L = L_1$ , or if  $L_1 \neq L_2$ , where  $L$  is the greater of  $L_1$  and  $L_2$ ;

and

wherein any base present may be substituted by an analogue thereof;

5        b)    mixing said cleavage means, said target nucleic acid, said first and second oligonucleotides to create a reaction mixture under reaction conditions such that at least said 5' portion of said first oligonucleotide is annealed to said target nucleic acid and wherein at least said 5' and central portion of said second oligonucleotide is annealed to said target nucleic acid so as to create a cleavage structure and wherein the combined melting temperature of said complementary regions within said 5' and 3' portions of said first oligonucleotide when annealed to said target nucleic acid is greater than the melting temperature of said 5' and central portion of said first oligonucleotide, and wherein cleavage of said cleavage structure occurs to generate non-target cleavage products; and

      c)    detecting said non-target cleavage products.

15        21.    The method of claim 20, wherein said reaction temperature is between approximately 50 and 70 degrees centigrade.

      22.    The method of claim 20, wherein said target nucleic acid molecules comprises single-stranded DNA.

20        23.    The method of claim 20, wherein said target nucleic acid molecules comprises double-stranded DNA and prior to step (c), said reaction mixture is treated such that said double-stranded DNA is rendered substantially single-stranded.

      24.    The method of claim 20, wherein said treatment to render said double-stranded DNA is rendered substantially single-stranded by increasing the temperature.

25        25.    The method of claim 20, wherein said target nucleic acid molecules comprises RNA and wherein said first and second oligonucleotide molecules comprise DNA.

      26.    The method of claim 20, wherein said cleavage means comprises a thermostable 5' nuclease.

30        27.    The method of claim 26, wherein a portion of the amino acid sequence is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

35        28.    The method of claim 28, wherein said organism is selected from the group consisting of *Thermus aquaticus*, *Thermus flavus* and *Thermus thermophilus*.

      29.    The method of claim 20, wherein said source of target nucleic acid molecules comprises a sample containing genomic DNA.

30. The method of claim 20, wherein said reaction conditions comprise providing a source of divalent cations.

31. The method of claim 30, wherein said divalent cation is selected from the group comprising  $Mn^{2+}$  and  $Mg^{2+}$  ions.

5 32. The method of claim 20, wherein the method includes a plurality of said target nucleic acid sequences and a plurality of said second oligonucleotide molecules such that each of said second oligonucleotide molecules has a distinct 3' region.

10 33. The method of claim 20, wherein the method includes at least ten said second oligonucleotide molecules or at least eleven said second oligonucleotide molecules, or at least twelve said second oligonucleotide molecules, or at least thirteen said second oligonucleotide molecules, or at least fourteen said second oligonucleotide molecules, or at least fifteen  
15 said second oligonucleotide molecules, or at least sixteen said second oligonucleotide molecules, or at least seventeen said second oligonucleotide molecules, or at least eighteen said second oligonucleotide molecules, or at least nineteen said second oligonucleotide molecules, or at least twenty said second oligonucleotide molecules, or at least twenty-one said second oligonucleotide molecules, or at least twenty-two said second oligonucleotide  
20 molecules, or at least twenty-three said second oligonucleotide molecules, or at least twenty-four said second oligonucleotide molecules, or at least twenty-five said second oligonucleotide molecules, or at least twenty-six said second oligonucleotide molecules, or at least twenty-seven said second oligonucleotide molecules, or at least twenty-eight said second  
25 oligonucleotide molecules, or at least twenty-nine said second oligonucleotide molecules, or at least thirty said second oligonucleotide molecules, or at least thirty-one said second oligonucleotide molecules, or at least thirty-two said second oligonucleotide molecules, or at least thirty-three said second oligonucleotide molecules, or at least thirty-four  
30 said second oligonucleotide molecules, or at least thirty-five said second oligonucleotide molecules, or at least thirty-six said second oligonucleotide molecules, or at least thirty-seven said second oligonucleotide molecules, or at least thirty-eight said second oligonucleotide molecules, or at least thirty-nine said second oligonucleotide molecules, or at least forty said  
35 second oligonucleotide molecules, or at least forty-one said second oligonucleotide molecules, or at least forty-two said second oligonucleotide molecules, or at least forty-three said second oligonucleotide molecules, or at least forty-four said second oligonucleotide molecules, or at least forty-

five said second oligonucleotide molecules, or at least forty-six said second oligonucleotide molecules, or at least forty-seven said second oligonucleotide molecules, or at least forty-eight said second oligonucleotide molecules, or at least forty-nine said second oligonucleotide molecules, or at least fifty said second oligonucleotide molecules, or at least sixty said second oligonucleotide molecules, or at least seventy said second oligonucleotide molecules, or at least eighty said second oligonucleotide molecules, or at least ninety said second oligonucleotide molecules, or at least one hundred said second oligonucleotide molecules, or at least one hundred and ten said second oligonucleotide molecules, or at least one hundred and twenty said second oligonucleotide molecules, or at least one hundred and thirty said second oligonucleotide molecules, or at least one hundred and forty said second oligonucleotide molecules, or at least one hundred and fifty said second oligonucleotide molecules, or at least one hundred and sixty said second oligonucleotide molecules, or at least one hundred and seventy said second oligonucleotide molecules, or at least one hundred and eighty said second oligonucleotide molecules, or at least one hundred and ninety said second oligonucleotide molecules, or at least two hundred said second oligonucleotide molecules.

20 34. The method of claim 20, wherein said 3' portion of said second oligonucleotide molecules incorporates a fluorescent molecule, a radiolabelled nucleotide, digoxigenin, biotinylation and the like.

35. A composition comprising a cleavage structure, said cleavage structure comprising:

25 a) a target nucleic acid having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region and said second region is located to and downstream from said third region;

30 b) a first oligonucleotide having a 5' portion and a 3' portion, said 5' portion of said first oligonucleotide having a sequence complementary to said second region of said target nucleic acid and said 3' portion of said first oligonucleotide having a sequence complementary to said third region of said target nucleic acid; and

35 c) a second oligonucleotide having a 5' portion, a central portion and a 3' portion, said 5' portion of said second oligonucleotide having a sequence complementary to said first region of said target nucleic acid, said central region of said second oligonucleotide having a sequence complementary to said second region of said target nucleic acid, and said 3' portion of

said second oligonucleotide having a sequence that is not base-paired to said target nucleic acid and is selected from a set of oligonucleotides based on a following group of sequences:

1 1 1 2 2 3 2 3 1 1 1 3 1 2 2 3 2 2 2 3 2 3 2 1  
3 2 2 1 3 1 3 2 2 1 1 2 2 3 2 1 2 2 2 3 1 2 3 1  
1 2 3 2 2 1 1 1 3 2 1 1 3 2 3 2 2 3 1 1 1 2 3 2  
2 3 1 2 3 2 2 1 3 1 1 3 2 1 2 1 2 2 3 2 3 1 1 2  
2 2 2 3 2 3 2 1 3 1 1 2 1 2 3 2 3 2 2 3 2 2 1 1  
1 2 1 1 3 2 3 2 1 1 3 2 3 1 1 1 2 1 1 3 1 1 3 1  
1 1 3 1 3 2 1 2 2 2 3 2 2 3 2 3 1 3 2 2 1 1 1 2  
3 2 3 2 2 2 1 2 3 2 2 1 2 1 2 3 2 3 1 1 3 2 2 2  
1 1 1 3 1 3 1 1 2 1 3 1 1 2 1 2 3 2 3 2 1 1 3 2  
2 1 2 3 1 1 1 3 1 3 2 3 1 3 1 2 1 1 2 3 2 2 2 1  
1 2 3 1 3 1 1 1 2 1 2 3 2 2 1 3 1 1 2 3 2 3 1 2  
2 2 1 3 2 2 3 2 2 3 1 2 3 2 2 2 1 3 2 1 3 2 2 2  
3 2 1 1 1 3 1 3 2 1 2 1 1 3 2 2 2 3 1 2 3 1 2 1  
1 1 1 3 2 1 1 3 1 1 2 3 1 2 3 2 1 1 2 1 1 3 2 3  
3 2 1 3 1 1 1 2 1 3 2 2 2 1 2 2 3 1 2 3 1 2 2 3  
2 3 2 1 1 3 2 3 1 1 1 2 1 3 2 3 1 3 2 2 1 2 2 2  
1 1 1 2 1 3 1 2 3 1 2 1 2 1 1 3 2 3 1 3 1 1 2 3  
1 2 1 1 3 2 2 1 2 1 1 3 2 3 2 2 1 2 3 2 3 1 3 2  
2 1 2 1 3 1 2 1 1 1 3 1 3 1 2 3 1 2 2 2 3 2 2 3  
1 3 1 3 2 2 3 1 3 1 1 2 3 2 1 2 1 3 2 1 2 2 1 2  
1 1 3 2 1 3 2 2 2 3 2 1 1 3 1 1 2 3 1 2 2 3 2 1  
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3 2 1 3 1 3 1 2 1 1 2 2 3 1 2 3 2 3 2 1 1 2 2 2

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wherein each of 1 to 3 is a nucleotide base selected to be different from the others of 1 to 3 with the proviso that up to three nucleotide bases of each sequence can be substituted with any nucleotide base

5 provided that:

for any pair of sequences of the set:

$M1 \leq 16$ ,  $M2 \leq 13$ ,  $M3 \leq 20$ ,  $M4 \leq 16$ , and  $M5 \leq 19$ , where:

M1 is the maximum number of matches for any alignment in which there are no internal indels;

M2 is the maximum length of a block of matches for any alignment;

M3 is the maximum number of matches for any alignment having a maximum score;

M4 is the maximum sum of the lengths of the longest two blocks of matches for any alignment of maximum score; and

M5 is the maximum sum of the lengths of all the blocks of matches having a length of at least 3, for any alignment of maximum score; wherein

the score of an alignment is determined according to the equation  $(A \times m) - (B \times mm) - (C \times (og + eg)) - (D \times eg)$ , wherein:

for each of (i) to (iv):

(i)  $m = 6$ ,  $mm = 6$ ,  $og = 0$  and  $eg = 6$ ,

(ii)  $m = 6$ ,  $mm = 6$ ,  $og = 5$  and  $eg = 1$ ,

(iii)  $m = 6$ ,  $mm = 2$ ,  $og = 5$  and  $eg = 1$ , and

(iv)  $m = 6$ ,  $mm = 6$ ,  $og = 6$  and  $eg = 0$ ,

A is the total number of matched pairs of bases in the alignment;

B is the total number of internal mismatched pairs in the alignment;

C is the total number of internal gaps in the alignment; and

D is the total number of internal indels in the alignment minus the total number of internal gaps in the alignment; and

wherein the maximum score is determined separately for each of (i), (ii), (iii) and (iv).

b) mixing said cleavage means, said target nucleic acid, said first and second oligonucleotides to create a reaction mixture under reaction conditions such that at least said 5' portion of said first oligonucleotide is annealed to said target nucleic acid and wherein at least said 5' and central portion of said second oligonucleotide is annealed to said target nucleic acid so as to create a cleavage structure and wherein the combined melting temperature of said complementary regions within said 5' and 3' portions of said first oligonucleotide when annealed to said target nucleic acid is greater than the melting temperature of said 5' and central portion of said first oligonucleotide, and wherein cleavage of said cleavage structure occurs to generate non-target cleavage products; and

c) detecting said non-target cleavage products.

36. The composition of claim 35, wherein one or more of said first and second oligonucleotides contain a 3'-terminal dideoxynucleotide.

5 37. The composition of claim 35, wherein the composition includes a plurality of said target nucleic acid molecules and a plurality of said second oligonucleotide molecules such that each of said second oligonucleotide molecules has a distinct 3' region.

10 38. The composition of claim 35, wherein the composition includes at least ten, or twenty, or thirty, or forty, or fifty, or sixty, or seventy, or eighty, or ninety, or one hundred, or one hundred and ten, or one hundred and twenty, or one hundred and thirty, or one hundred and forty, or one hundred and fifty, or one hundred and sixty said second oligonucleotide molecules, or comprising one hundred and seventy said second oligonucleotide molecules, or  
15 comprising one hundred and eighty said second oligonucleotide molecules, or comprising one hundred and ninety said second oligonucleotide molecules, or comprising two hundred said second oligonucleotide molecules, or comprising two hundred and twenty said second oligonucleotide molecules, or comprising two hundred and forty said second oligonucleotide molecules, or comprising two hundred and sixty said second oligonucleotide molecules, or comprising  
20 two hundred and eighty said second oligonucleotide molecules, or comprising three hundred said second oligonucleotide molecules, or comprising four hundred said second oligonucleotide molecules, or comprising five hundred said second oligonucleotide molecules, or comprising six hundred said second oligonucleotide molecules, or comprising seven hundred said second  
25 oligonucleotide molecules, or comprising eight hundred said second oligonucleotide molecules, or comprising nine hundred said second oligonucleotide molecules, or comprising one thousand said second oligonucleotide molecules, or comprising eleven hundred said second oligonucleotide molecules.

30 39. A method of detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products, the method comprising:

a) providing:

i) a cleavage means,

35 ii) a target nucleic acid, said target nucleic acid having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region, and said second region is located adjacent to and downstream from said third region;

iii) a first oligonucleotide having a 5' and a 3' portion, said 5' portion of said first oligonucleotide having a sequence complementary to said second region of said target nucleic acid and said 3' portion of said first oligonucleotide having a sequence complementary to said third region of said target nucleic acid;

iv) a second oligonucleotide having a 5' portion, a central portion and a 3' portion, said 5' portion of said second oligonucleotide having a sequence complementary to said first region of said target nucleic acid, said central portion of said second oligonucleotide having a sequence complimentary to said second region of said target nucleic acid, and said 3' portion of said second oligonucleotide having a sequence that is not base-paired to said target nucleic acid and is selected from a set of oligonucleotides based on a following group of sequences,

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- wherein each of 1 to 3 is a nucleotide base selected to be different from the others of 1 to 3 with the proviso that up to three nucleotide bases of
- 5 each sequence can be substituted with any nucleotide base provided that:

for any pair of sequences of the set:

$M1 \leq 16$ ,  $M2 \leq 13$ ,  $M3 \leq 20$ ,  $M4 \leq 16$ , and  $M5 \leq 19$ , where:

$M1$  is the maximum number of matches for any alignment in which there are no internal indels;

$M2$  is the maximum length of a block of matches for any alignment;

$M3$  is the maximum number of matches for any alignment having a maximum score;

$M4$  is the maximum sum of the lengths of the longest two blocks of matches for any alignment of maximum score; and

$M5$  is the maximum sum of the lengths of all the blocks of matches having a length of at least 3, for any alignment of maximum score;

wherein:

the score of an alignment is determined according to the equation  $(A \times m) - (B \times mm) - (C \times (og + eg)) - (D \times eg)$ , wherein:

for each of (i) to (iv):

(i)  $m = 6$ ,  $mm = 6$ ,  $og = 0$  and  $eg = 6$ ,

(ii)  $m = 6$ ,  $mm = 6$ ,  $og = 5$  and  $eg = 1$ ,

(iii)  $m = 6$ ,  $mm = 2$ ,  $og = 5$  and  $eg = 1$ , and

(iv)  $m = 6$ ,  $mm = 6$ ,  $og = 6$  and  $eg = 0$ ,

$A$  is the total number of matched pairs of bases in the alignment;

$B$  is the total number of internal mismatched pairs in the alignment;

$C$  is the total number of internal gaps in the alignment; and

$D$  is the total number of internal indels in the alignment minus the total number of internal gaps in the alignment; and

wherein the maximum score is determined separately for each of (i),

(ii), (iii) and (iv).

b) mixing said cleavage means, said target nucleic acid, said first and second oligonucleotides to create a reaction mixture under reaction conditions such that at least said 5' portion of said first oligonucleotide is annealed to said target nucleic acid and wherein at least said 5' and  
5 central portion of said second oligonucleotide is annealed to said target nucleic acid so as to create a cleavage structure and wherein the combined melting temperature of said complementary regions within said 5' and 3' portions of said first oligonucleotide when annealed to said target nucleic acid is greater than the melting temperature of said 5' and central portion  
10 of said first oligonucleotide, and wherein cleavage of said cleavage structure occurs to generate non-target cleavage products; and

c) detecting said non-target cleavage products.

40. The composition of claim 39, wherein one or more of said first and second oligonucleotides contain a 3'-terminal dideoxynucleotide.

15 41. The composition of claim 39, wherein the composition includes a plurality of said target nucleic acid sequences and a plurality of said second oligonucleotide molecules such that each of said second oligonucleotide molecules has a distinct 3' region.

43. The composition of claim 39, wherein the composition includes at  
20 least ten, or twenty, or thirty, or forty, or fifty, or sixty, or seventy, or eighty, or ninety, or one hundred, or one hundred and ten, or one hundred and twenty, or one hundred and thirty, or one hundred and forty, or one hundred and fifty, or one hundred and sixty said second oligonucleotide molecules, or  
25 comprising one hundred and seventy said second oligonucleotide molecules, or comprising one hundred and eighty said second oligonucleotide molecules, or comprising one hundred and ninety said second oligonucleotide molecules, or comprising two hundred said second oligonucleotide molecules, or comprising two hundred and twenty said second oligonucleotide molecules, or comprising two hundred and forty said second oligonucleotide molecules, or comprising  
30 two hundred and sixty said second oligonucleotide molecules, or comprising two hundred and eighty said second oligonucleotide molecules, or comprising three hundred said second oligonucleotide molecules, or comprising four hundred said second oligonucleotide molecules, or comprising five hundred said second oligonucleotide molecules, or comprising six hundred said second  
35 oligonucleotide molecules, or comprising seven hundred said second oligonucleotide molecules, or comprising eight hundred said second oligonucleotide molecules, or comprising nine hundred said second oligonucleotide molecules, or comprising one thousand said second



oligonucleotide molecules, or comprising eleven hundred said second oligonucleotide molecules.

44. A method of analyzing a biological sample comprising a plurality of target nucleic acid molecules for the presence of a mutation or polymorphism at a locus of each target nucleic acid molecule, the method comprising:

a) providing:

i) a cleavage means,

ii) a plurality of target nucleic acid molecules, each of said target nucleic acid molecules having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region, and said second region is located adjacent to and downstream from said third region;

iii) a plurality of first oligonucleotide molecules, each having a 5' and a 3' portion, said 5' portion of said first oligonucleotide molecules having a sequence complementary to said second region of said target nucleic acid molecules and said 3' portion of said first oligonucleotide molecules having a sequence complementary to said third region of said target nucleic acid molecules;

iv) a plurality of second oligonucleotide molecules, each having a 5' portion, a central portion and a 3' portion, said 5' portion of said second oligonucleotide molecules having a sequence complementary to said first region of said target nucleic acid molecules, said central portion of said second oligonucleotide molecules having a sequence complimentary to said second region of said target nucleic acid molecules, and said 3' portion of said second oligonucleotide molecules having a sequence that is not base-paired to said target nucleic acid and is selected from a set of oligonucleotides based on a following group of sequences,

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wherein each of 1 to 3 is a nucleotide base selected to be different from the others of 1 to 3 with the proviso that up to three nucleotide bases of each sequence can be substituted with any nucleotide base provided that:

for any pair of sequences of the set:

$M1 \leq 16$ ,  $M2 \leq 13$ ,  $M3 \leq 20$ ,  $M4 \leq 16$ , and  $M5 \leq 19$ , where:

M1 is the maximum number of matches for any alignment in which there are no internal indels;

M2 is the maximum length of a block of matches for any alignment;

M3 is the maximum number of matches for any alignment having a maximum score;

M4 is the maximum sum of the lengths of the longest two blocks of matches for any alignment of maximum score; and

M5 is the maximum sum of the lengths of all the blocks of matches having a length of at least 3, for any alignment of maximum score;

wherein:

the score of an alignment is determined according to the equation  $(A \times m) - (B \times mm) - (C \times (og + eg)) - (D \times eg)$ , wherein:

for each of (i) to (iv):



- (i)  $m = 6$ ,  $mm = 6$ ,  $og = 0$  and  $eg = 6$ ,
- (ii)  $m = 6$ ,  $mm = 6$ ,  $og = 5$  and  $eg = 1$ ,
- (iii)  $m = 6$ ,  $mm = 2$ ,  $og = 5$  and  $eg = 1$ , and
- (iv)  $m = 6$ ,  $mm = 6$ ,  $og = 6$  and  $eg = 0$ ,

A is the total number of matched pairs of bases in the alignment;

B is the total number of internal mismatched pairs in the alignment;

C is the total number of internal gaps in the alignment; and

D is the total number of internal indels in the alignment minus the total number of internal gaps in the alignment; and

wherein the maximum score is determined separately for each of (i), (ii), (iii) and (iv).

b) mixing said cleavage means, said target nucleic acid, said first and second oligonucleotides to create a reaction mixture under reaction conditions such that at least said 5' portion of said first oligonucleotide is annealed to said target nucleic acid and wherein at least said 5' and central portion of said second oligonucleotide is annealed to said target nucleic acid so as to create a cleavage structure and wherein the combined melting temperature of said complementary regions within said 5' and 3' portions of said first oligonucleotide when annealed to said target nucleic acid is greater than the melting temperature of said 5' and central portion of said first oligonucleotide, and wherein cleavage of said cleavage structure occurs to generate non-target cleavage products; and

c) detecting said non-target cleavage products.

45. The method of claim 44, wherein said reaction temperature is between approximately 50 and 70 degrees centigrade.

46. The method of claim 44; wherein said target nucleic acid molecules comprises single-stranded DNA.

47. The method of claim 44, wherein said target nucleic acid molecules comprises double-stranded DNA and prior to step (c), said reaction mixture is treated such that said double-stranded DNA is rendered substantially single-stranded.

48. The method of claim 47, wherein said treatment to render said double-stranded DNA is rendered substantially single-stranded by increasing the temperature.

49. The method of claim 44, wherein said target nucleic acid molecules comprises RNA and wherein said first and second oligonucleotide molecules comprise DNA.

50. The method of claim 44, wherein said cleavage means comprises a  
5 thermostable 5' nuclease.

51. The method of claim 50, wherein a portion of the amino acid sequence is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

52. The method of claim 51, wherein said organism is selected from  
10 the group consisting of *Thermus aquaticus*, *Thermus flavus* and *Thermus thermophilus*.

53. The method of claim 44, wherein said source of target nucleic acid molecules comprises a sample containing genomic DNA.

54. The method of claim 44, wherein said reaction conditions comprise  
15 providing a source of divalent cations.

- 5            55.    A method of detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products, the method comprising:
- a)    providing:
- i)    a cleavage means,
- ii)   a first target nucleic acid, said first target nucleic acid
- 10    having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region, and said second region is located adjacent to and downstream from said third region;
- iii) a first oligonucleotide having a 5' and a 3' portion, said 5'
- 15    portion of said first oligonucleotide having a sequence complementary to said second region of said target nucleic acid and said 3' portion of said first oligonucleotide having a sequence complementary to said third region of said target nucleic acid;
- iv) a second oligonucleotide having a 5' portion, a central portion
- 20    and a 3' portion, said 5' portion of said second oligonucleotide having a sequence complementary to said first region of said target nucleic acid, said central portion of said second oligonucleotide having a sequence complimentary to said second region of said target nucleic acid, and said 3'
- 25    portion of said second oligonucleotide having a sequence that is not base-paired to said target nucleic acid and is selected from a set of oligonucleotides, based on a following group of sequences each having a 3' and 5' portion,

1	4	6	6	1	3
2	4	5	5	2	3
1	8	1	2	3	4
1	7	1	9	8	4
1	1	9	2	6	9
1	2	4	3	9	6
9	8	9	8	10	9
9	1	2	3	8	10
8	8	7	4	3	1
1	1	1	1	1	2
2	1	3	3	2	2
3	1	2	2	3	2
4	1	4	4	4	2
1	2	3	3	1	1

1	3	2	2	1	4
3	3	3	3	3	4
4	3	1	1	4	4
3	4	1	1	3	3
3	6	6	6	3	5
6	6	1	1	6	5
7	6	7	7	7	5
8	7	5	5	8	8
2	1	7	7	1	1
2	3	2	3	1	3
2	6	5	6	1	6
4	8	1	1	3	8
5	3	1	1	6	3
5	6	8	8	6	6
8	3	6	5	7	3
1	2	3	1	4	6
1	5	7	5	4	3
2	1	6	7	3	6
2	6	1	3	3	1
2	7	6	8	3	1
3	4	3	1	2	5
3	5	6	1	2	7
3	6	1	7	2	7
4	6	3	5	1	7
5	4	6	3	8	6
6	8	2	3	7	1
7	1	7	8	6	3
7	3	4	1	6	8
4	7	7	1	2	4
3	6	5	2	6	3
1	4	1	4	6	1
3	3	1	4	8	1
8	3	3	5	3	8
1	3	6	6	3	7
7	3	8	6	4	7
3	1	3	7	8	6
10	9	5	5	10	10
7	10	10	10	7	9

9	9	7	7	10	9
9	3	10	3	10	3
9	6	3	4	10	6
10	4	10	3	9	4
3	9	3	10	4	9
9	10	5	9	4	8
3	9	4	9	10	7
3	5	9	4	10	8
4	10	5	4	9	3
5	3	3	9	8	10
6	8	6	9	7	10
4	6	10	9	6	4
4	9	8	10	8	3
7	7	9	10	5	3
8	8	9	3	9	10
8	10	2	9	5	9
9	6	2	2	7	10
9	7	5	3	10	6
10	3	6	8	9	2
10	9	3	2	7	3
8	9	10	3	6	2
3	2	5	10	8	9
8	2	3	10	2	9
6	3	9	8	2	10
3	7	3	9	9	10
9	10	1	1	9	4
10	1	9	1	4	1
7	1	10	9	8	1
9	1	10	1	10	6
9	6	9	1	3	10
3	10	8	8	9	1
3	8	1	9	10	3
9	10	1	3	6	9
1	9	1	10	3	1
1	4	9	6	8	10
3	3	9	6	1	10
5	3	1	6	9	10
6	1	8	10	9	6

5	9	9	4	10	3
2	10	9	1	9	5
10	10	7	2	1	9
10	9	9	1	8	2
1	8	6	8	9	10
1	9	1	3	8	10
9	6	9	10	1	2
1	10	8	9	9	2
1	9	6	7	2	9
4	3	9	3	5	1
5	11	10	14	12	1
7	12	4	13	3	2
5	5	4	4	12	9
2	13	13	11	13	13
10	2	5	4	12	7
11	7	4	11	6	4
12	12	1	9	11	11
12	9	4	14	12	6
12	7	13	2	9	11
9	11	3	4	1	3
10	5	12	11	4	4
4	13	7	12	1	5
9	13	10	11	11	6
10	14	14	10	1	3
2	14	1	10	4	5
10	12	12	7	11	10
9	11	2	12	8	11
2	8	5	2	12	14
1	8	13	3	7	8
9	4	7	5	4	2
13	2	12	7	1	12
11	10	9	7	5	11
8	12	2	2	12	7
5	2	14	3	4	13
1	8	8	1	5	9
14	5	11	10	13	3
14	1	4	13	2	4
4	4	5	11	3	10

10	9	2	3	3	11
11	4	8	14	3	4
5	1	14	8	11	2
14	3	11	6	12	5
13	4	4	1	10	1
6	10	11	6	5	1
5	8	12	5	1	7
4	5	9	6	9	2
13	2	4	4	2	3
11	2	2	5	9	3
8	1	10	12	2	8
12	7	9	11	4	1
12	1	4	14	3	13
11	2	7	10	4	1
3	4	12	11	11	11
3	3	4	2	12	11
1	5	9	4	2	1
6	1	12	2	10	5
10	5	1	12	2	14
2	11	7	9	4	11
7	4	4	5	14	12
12	5	2	1	10	12
5	9	2	11	6	1
12	14	3	6	1	14
5	9	11	10	1	4
2	5	12	14	10	10
4	5	8	4	5	6
10	12	4	6	12	5
4	2	1	13	6	8
9	10	10	14	5	3
6	14	10	11	3	3
2	9	10	12	5	7
13	3	7	10	5	12
6	4	1	2	5	13
6	1	13	4	14	13
2	12	1	14	1	9
4	11	13	2	6	10
1	10	7	4	5	8

7	2	2	10	13	4
8	2	11	4	6	14
4	8	2	6	2	3
7	1	12	11	2	9
5	6	10	4	13	4
5	10	4	11	9	3
3	11	9	3	2	3
8	15	6	20	17	19
21	10	15	3	7	11
11	7	17	20	14	9
16	6	17	13	21	21
10	15	22	6	17	21
15	7	17	10	22	22
3	20	8	15	20	16
17	21	10	16	6	22
6	21	14	14	14	16
7	17	3	20	10	7
16	19	14	17	7	21
20	16	7	15	22	10
20	10	18	11	22	18
18	7	19	15	7	22
21	18	7	21	16	3
14	13	7	22	17	13
19	7	8	12	10	17
15	3	21	14	9	7
19	6	15	7	14	14
4	17	10	15	20	19
21	6	18	4	20	16
2	19	8	17	6	13
12	12	6	17	4	20
16	21	12	10	19	16
14	14	15	2	7	21
8	16	21	6	22	16
14	17	22	14	17	20
10	21	7	15	21	18
16	13	20	18	21	12
15	7	4	22	14	13
7	19	14	8	15	4



4	5	3	20	7	16
22	18	6	18	13	20
19	6	16	3	13	3
18	6	22	7	20	18
10	17	11	21	8	13
7	10	17	19	10	14

5

wherein:

- (A) each of 1 to 22 is a 4mer selected from the group of 4mers consisting of WWWW, WWWX, WWY, WXXW, WWXX, WWXY, WWYW, WWYX, WWYY, WXWW, WXWX, WXWY, WXXW, WXXX, WXXY, WXYW, WXYX, WXY, WYWW, WYWX, WYWY, WYXW, WYXX, WYXY, WYYW, WYYX, WYYY, XWWW, XWWX, XWWY, XWXW, XWXX, XWXY, XWYW, XWYX, XWYY, XXWW, XXWX, XXWY, XXXW, XXXX, XXXY, XXYW, XXYX, XXYY, XYWW, XYWX, XYWY, XYXW, XYXX, XYY, XYYW, XYYX, XYYY, YWWW, YWWX, YWWY, YWXW, YWXX, YWXY, YWYW, YWYX, YWYY, YXWW, YXWX, YXWY, YXXW, YXXX, YXXY, YXYW, YXYX, YXY, YYWW, YYWX, YYWY, YYXW, YYXX, YXXY, YYYW, YYYX, and YYYY, and
- (B) each of 1 to 22 is selected so as to be different from all of the others of 1 to 22;
- (C) each of W, X and Y is a base in which:
- (i) (a) W = one of A, T/U, G, and C,  
X = one of A, T/U, G, and C,  
Y = one of A, T/U, G, and C,  
and each of W, X and Y is selected so as to be different from all of the others of W, X and Y,
  - (b) an unselected said base of (i)(a) can be substituted any number of times for any one of W, X and Y, or
  - (ii) (a) W = G or C,  
X = A or T/U,  
Y = A or T/U,  
and X ≠ Y, and
  - (b) a base not selected in (ii)(a) can be inserted into each sequence at one or more locations, the location of each insertion being the same in all the sequences;
- (D) up to three bases can be inserted at any location of any of the sequences or up to three bases can be deleted from any of the sequences;
- (E) all of the sequences of a said group of oligonucleotides are read 5'

to 3' or are read 3' to 5'; and wherein each oligonucleotide of a said set has a sequence of at least ten contiguous bases of the sequence on which it is based, provided that:

(F) (I) the quotient of the sum of G and C divided by the sum of A, T/U, G and C for all combined sequences of the set is between about 0.1 and 0.40 and said quotient for each sequence of the set does not vary from the quotient for the combined sequences by more than 0.2; and

(II) for any phantom sequence generated from any pair of first and second sequences of the set  $L_1$  and  $L_2$  in length, respectively, by selection from the first and second sequences of identical bases in identical sequence with each other:

- (i) any consecutive sequence of bases in the phantom sequence which is identical to a consecutive sequence of bases in each of the first and second sequences from which it is generated is less than  $((3/4 \times L) - 1)$  bases in length;
- (ii) the phantom sequence, if greater than or equal to  $(5/6 \times L)$  in length, contains at least three insertions/deletions or mismatches when compared to the first and second sequences from which it is generated; and
- (iii) the phantom sequence is not greater than or equal to  $(11/12 \times L)$  in length;

where  $L = L_1$ , or if  $L_1 \neq L_2$ , where  $L$  is the greater of  $L_1$  and  $L_2$ ; and

5 wherein any base present may be substituted by an analogue thereof;

v) a second target nucleic acid, distinct from said first target nucleic acid, and having a fourth region, a fifth region and a sixth region, wherein said fourth region is located adjacent to and downstream from said fifth region, and said fifth region is located adjacent to and downstream from said sixth region, said fifth region having a sequence complementary to said 3' portion of said sequence selected from the group of sequences listed in step (a)(iv), said sixth region having a sequence complementary to said 5' portion of the sequence selected from the group of sequences in step (a)(iv);

15 vi) a third oligonucleotide having a 5' portion, a central portion and a 3' portion, said 5' portion of said third oligonucleotide

- 5 having a sequence complementary to said fourth region of said second target nucleic acid, said central portion of said third oligonucleotide having a sequence complementary to said fifth region of said second target nucleic acid, and said 3' portion of said third oligonucleotide having a sequence that is not base paired to either said second  
10 target nucleic acid or said first target nucleic acid and is selected from a set of oligonucleotides based on the group of sequences listed in step (a) (iv) such that said sequence selected is distinct from said sequence selected in step (a) (iv);
- b) mixing said cleavage means, said first target nucleic acid, said  
15 second target nucleic acid, said first, second, and third oligonucleotides to create a reaction mixture under reaction conditions such that at least said 5' portion of said first oligonucleotide is annealed to said first target nucleic acid and wherein at least said 5' and central portion of said second oligonucleotide is annealed to said first target nucleic acid so as to create  
20 a first cleavage structure and wherein the combined melting temperature of said complementary regions within said 5' and 3' portions of said first oligonucleotide when annealed to said first target nucleic acid is greater than the melting temperature of said 5' and central portion of said first oligonucleotide, wherein cleavage of said first cleavage structure occurs to  
25 generate a first non-target cleavage product, and wherein at least said 5' portion first non-target cleavage product is annealed to said second target nucleic acid and at least said 5' and central portion of said third oligonucleotide is annealed to said second target nucleic acid so as to create a second cleavage structure and wherein the combined melting  
30 temperature of said complementary regions within said 5' and 3' portions of said non-target cleavage product when annealed to said second target nucleic acid is greater than the melting temperature of said 5' and central portion of said third oligonucleotide, wherein cleavage of said second cleavage structure occurs to generate a second non-target cleavage product; and
- 35 c) detecting said second non-target cleavage product.
56. The method of claim 55, wherein said first target nucleic acid is genomic DNA and said second target nucleic acid is synthetic DNA.
57. The method of claim 55, wherein said synthetic DNA has at  
40 least one hairpin loop.
58. The method of claim 57, wherein the method includes a plurality of said first target nucleic acid sequences, a plurality of first

5 oligonucleotide molecules, a plurality of said second oligonucleotide molecules, a plurality of said second target nucleic acid sequences and a plurality of third oligonucleotide molecules.

59. A method of analyzing a biological sample comprising a plurality of target nucleic acid molecules for the presence of a mutation or  
10 polymorphism at a locus of each target nucleic acid molecule, the method comprising:

a) providing:

i) a cleavage means,

15 ii) a first target nucleic acid, said first target nucleic acid having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region, and said second region is located adjacent to and downstream from said third region;

20 iii) a first oligonucleotide having a 5' and a 3' portion, said 5' portion of said first oligonucleotide having a sequence complementary to said second region of said target nucleic acid and said 3' portion of said first oligonucleotide having a sequence complementary to said third region of said target nucleic acid;

25 iv) a second oligonucleotide having a 5' portion, a central portion and a 3' portion, said 5' portion of said second oligonucleotide having a sequence complementary to said first region of said target nucleic acid, said central portion of said second oligonucleotide having a sequence complimentary to said second region of said target nucleic acid, and said 3' portion of said second oligonucleotide having a sequence that is not base-  
30 paired to said target nucleic acid and is selected from a set of oligonucleotides, based on a following group of sequences each having a 3' and 5' portion,

1	4	6	6	1	3
2	4	5	5	2	3
1	8	1	2	3	4
1	7	1	9	8	4
1	1	9	2	6	9
1	2	4	3	9	6
9	8	9	8	10	9
9	1	2	3	8	10
8	8	7	4	3	1

1	1	1	1	1	2
2	1	3	3	2	2
3	1	2	2	3	2
4	1	4	4	4	2
1	2	3	3	1	1
1	3	2	2	1	4
3	3	3	3	3	4
4	3	1	1	4	4
3	4	1	1	3	3
3	6	6	6	3	5
6	6	1	1	6	5
7	6	7	7	7	5
8	7	5	5	8	8
2	1	7	7	1	1
2	3	2	3	1	3
2	6	5	6	1	6
4	8	1	1	3	8
5	3	1	1	6	3
5	6	8	8	6	6
8	3	6	5	7	3
1	2	3	1	4	6
1	5	7	5	4	3
2	1	6	7	3	6
2	6	1	3	3	1
2	7	6	8	3	1
3	4	3	1	2	5
3	5	6	1	2	7
3	6	1	7	2	7
4	6	3	5	1	7
5	4	6	3	8	6
6	8	2	3	7	1
7	1	7	8	6	3
7	3	4	1	6	8
4	7	7	1	2	4
3	6	5	2	6	3
1	4	1	4	6	1
3	3	1	4	8	1
8	3	3	5	3	8

1	3	6	6	3	7
7	3	8	6	4	7
3	1	3	7	8	6
10	9	5	5	10	10
7	10	10	10	7	9
9	9	7	7	10	9
9	3	10	3	10	3
9	6	3	4	10	6
10	4	10	3	9	4
3	9	3	10	4	9
9	10	5	9	4	8
3	9	4	9	10	7
3	5	9	4	10	8
4	10	5	4	9	3
5	3	3	9	8	10
6	8	6	9	7	10
4	6	10	9	6	4
4	9	8	10	8	3
7	7	9	10	5	3
8	8	9	3	9	10
8	10	2	9	5	9
9	6	2	2	7	10
9	7	5	3	10	6
10	3	6	8	9	2
10	9	3	2	7	3
8	9	10	3	6	2
3	2	5	10	8	9
8	2	3	10	2	9
6	3	9	8	2	10
3	7	3	9	9	10
9	10	1	1	9	4
10	1	9	1	4	1
7	1	10	9	8	1
9	1	10	1	10	6
9	6	9	1	3	10
3	10	8	8	9	1
3	8	1	9	10	3
9	10	1	3	6	9

1	9	1	10	3	1
1	4	9	6	8	10
3	3	9	6	1	10
5	3	1	6	9	10
6	1	8	10	9	6
5	9	9	4	10	3
2	10	9	1	9	5
10	10	7	2	1	9
10	9	9	1	8	2
1	8	6	8	9	10
1	9	1	3	8	10
9	6	9	10	1	2
1	10	8	9	9	2
1	9	6	7	2	9
4	3	9	3	5	1
5	11	10	14	12	1
7	12	4	13	3	2
5	5	4	4	12	9
2	13	13	11	13	13
10	2	5	4	12	7
11	7	4	11	6	4
12	12	1	9	11	11
12	9	4	14	12	6
12	7	13	2	9	11
9	11	3	4	1	3
10	5	12	11	4	4
4	13	7	12	1	5
9	13	10	11	11	6
10	14	14	10	1	3
2	14	1	10	4	5
10	12	12	7	11	10
9	11	2	12	8	11
2	8	5	2	12	14
1	8	13	3	7	8
9	4	7	5	4	2
13	2	12	7	1	12
11	10	9	7	5	11
8	12	2	2	12	7

5	2	14	3	4	13
1	8	8	1	5	9
14	5	11	10	13	3
14	1	4	13	2	4
4	4	5	11	3	10
10	9	2	3	3	11
11	4	8	14	3	4
5	1	14	8	11	2
14	3	11	6	12	5
13	4	4	1	10	1
6	10	11	6	5	1
5	8	12	5	1	7
4	5	9	6	9	2
13	2	4	4	2	3
11	2	2	5	9	3
8	1	10	12	2	8
12	7	9	11	4	1
12	1	4	14	3	13
11	2	7	10	4	1
3	4	12	11	11	11
3	3	4	2	12	11
1	5	9	4	2	1
6	1	12	2	10	5
10	5	1	12	2	14
2	11	7	9	4	11
7	4	4	5	14	12
12	5	2	1	10	12
5	9	2	11	6	1
12	14	3	6	1	14
5	9	11	10	1	4
2	5	12	14	10	10
4	5	8	4	5	6
10	12	4	6	12	5
4	2	1	13	6	8
9	10	10	14	5	3
6	14	10	11	3	3
2	9	10	12	5	7
13	3	7	10	5	12



6	4	1	2	5	13
6	1	13	4	14	13
2	12	1	14	1	9
4	11	13	2	6	10
1	10	7	4	5	8
7	2	2	10	13	4
8	2	11	4	6	14
4	8	2	6	2	3
7	1	12	11	2	9
5	6	10	4	13	4
5	10	4	11	9	3
3	11	9	3	2	3
8	15	6	20	17	19
21	10	15	3	7	11
11	7	17	20	14	9
16	6	17	13	21	21
10	15	22	6	17	21
15	7	17	10	22	22
3	20	8	15	20	16
17	21	10	16	6	22
6	21	14	14	14	16
7	17	3	20	10	7
16	19	14	17	7	21
20	16	7	15	22	10
20	10	18	11	22	18
18	7	19	15	7	22
21	18	7	21	16	3
14	13	7	22	17	13
19	7	8	12	10	17
15	3	21	14	9	7
19	6	15	7	14	14
4	17	10	15	20	19
21	6	18	4	20	16
2	19	8	17	6	13
12	12	6	17	4	20
16	21	12	10	19	16
14	14	15	2	7	21
8	16	21	6	22	16

14	17	22	14	17	20
10	21	7	15	21	18
16	13	20	18	21	12
15	7	4	22	14	13
7	19	14	8	15	4
4	5	3	20	7	16
22	18	6	18	13	20
19	6	16	3	13	3
18	6	22	7	20	18
10	17	11	21	8	13
7	10	17	19	10	14

5

wherein:

- (A) each of 1 to 22 is a 4mer selected from the group of 4mers consisting of WWWW, WWWX, WWXY, WWXW, WWXX, WWXY, WWYW, WWYX, WWYY, WXWW, WXWX, WXWY, WXXW, WXXX, WXXY, WXYW, WXYX, WXYX, WXYX, WYWW, WYWX, WYWY, WYXW, WYXX, WYXY, WYYW, WYYX, WYYY, XWWW, XWWX, XWWY, XWXW, XWXX, XWXY, XWYW, XWYX, XWYY, XXWW, XXWX, XXWY, XXXW, XXXX, XXXY, XXYW, XXYX, XXYX, XYYW, XYWW, XYWX, XYWY, XYXW, XYXX, XYXY, XYYW, XYYX, XYYY, YWWW, YWWX, YWWY, YWXW, YWXX, YWXY, YWYW, YWYX, YWYY, YXWW, YXWX, YXWY, YXXW, YXXX, YXXY, YXYW, YXYX, YXYX, YYYW, YYWX, YYWY, YYXW, YYXX, YYYX, YYYW, YYYX, and YYYY, and
- (B) each of 1 to 22 is selected so as to be different from all of the others of 1 to 22;
- (C) each of W, X and Y is a base in which:
- (i) (a) W = one of A, T/U, G, and C,  
X = one of A, T/U, G, and C,  
Y = one of A, T/U, G, and C,  
and each of W, X and Y is selected so as to be different from all of the others of W, X and Y,
  - (b) an unselected said base of (i) (a) can be substituted any number of times for any one of W, X and Y, or
  - (ii) (a) W = G or C,  
X = A or T/U,  
Y = A or T/U,  
and X ≠ Y, and
  - (b) a base not selected in (ii) (a) can be inserted into each sequence at one or more locations, the location of each

insertion being the same in all the sequences;

- (D) up to three bases can be inserted at any location of any of the sequences or up to three bases can be deleted from any of the sequences;
- (E) all of the sequences of a said group of oligonucleotides are read 5' to 3' or are read 3' to 5'; and

wherein each oligonucleotide of a said set has a sequence of at least ten contiguous bases of the sequence on which it is based, provided that:

- (F) (I) the quotient of the sum of G and C divided by the sum of A, T/U, G and C for all combined sequences of the set is between about 0.1 and 0.40 and said quotient for each sequence of the set does not vary from the quotient for the combined sequences by more than 0.2; and
- (II) for any phantom sequence generated from any pair of first and second sequences of the set  $L_1$  and  $L_2$  in length, respectively, by selection from the first and second sequences of identical bases in identical sequence with each other:
  - (i) any consecutive sequence of bases in the phantom sequence which is identical to a consecutive sequence of bases in each of the first and second sequences from which it is generated is less than  $((3/4 \times L) - 1)$  bases in length;
  - (ii) the phantom sequence, if greater than or equal to  $(5/6 \times L)$  in length, contains at least three insertions/deletions or mismatches when compared to the first and second sequences from which it is generated; and
  - (iii) the phantom sequence is not greater than or equal to  $(11/12 \times L)$  in length;

where  $L = L_1$ , or if  $L_1 \neq L_2$ , where  $L$  is the greater of  $L_1$  and  $L_2$ ; and

5 wherein any base present may be substituted by an analogue thereof;

- v) a second target nucleic acid, distinct from said first target nucleic acid, and having a fourth region, a fifth region and a sixth region, wherein said fourth region is located adjacent to and downstream from said fifth region, and said fifth region is located adjacent to and downstream from said sixth region, said fifth region having a sequence complementary to said 3' portion of said sequence
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5 selected from the group of sequences listed in step (a)(iv), said sixth region having a sequence complementary to said 5' portion of the sequence selected from the group of sequences in step (a)(iv);

10 vi) a third oligonucleotide having a 5' portion, a central portion and a 3' portion, said 5' portion of said third oligonucleotide having a sequence complementary to said fourth region of said second target nucleic acid, said central portion of said third oligonucleotide having a sequence complementary to said fifth region of said second target nucleic acid, and said 3' portion of said third oligonucleotide having a sequence that is not base paired to either said second  
15 target nucleic acid or said first target nucleic acid and is selected from a set of oligonucleotides based on the group of sequences listed in step (a)(iv) such that said sequence selected is distinct from said sequence selected in step (a)(iv); and

20 b) mixing said cleavage means, said first target nucleic acid, said second target nucleic acid, said first, second, and third oligonucleotides to create a reaction mixture under reaction conditions such that at least said 5' portion of said first oligonucleotide is annealed to said first target nucleic acid and wherein at least said 5' and central portion of said second oligonucleotide is annealed to said first target nucleic acid so as to create  
25 a first cleavage structure and wherein the combined melting temperature of said complementary regions within said 5' and 3' portions of said first oligonucleotide when annealed to said first target nucleic acid is greater than the melting temperature of said 5' and central portion of said first oligonucleotide, wherein cleavage of said first cleavage structure occurs to  
30 generate a first non-target cleavage product, and wherein at least said 5' portion first non-target cleavage product is annealed to said second target nucleic acid and at least said 5' and central portion of said third oligonucleotide is annealed to said second target nucleic acid so as to create a second cleavage structure and wherein the combined melting  
35 temperature of said complementary regions within said 5' and 3' portions of said non-target cleavage product when annealed to said second target nucleic acid is greater than the melting temperature of said 5' and central portion of said third oligonucleotide, wherein cleavage of said second cleavage structure occurs to generate a second non-target cleavage product; and

40 c) detecting said second non-target cleavage product.

- 5           60. The method of claim 59, wherein said first target nucleic acid is genomic DNA and said second target nucleic acid is synthetic DNA.
61. The method of claim 60, wherein said synthetic DNA has at least one hairpin loop.
- 10          62. The method of claim 61, wherein the method includes a plurality of said first target nucleic acid sequences, a plurality of first oligonucleotide molecules, a plurality of said second oligonucleotide molecules, a plurality of said second target nucleic acid sequences and a plurality of third oligonucleotide molecules.
- 15          63. A method of detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products, the method comprising:
- a) providing:
- i) a cleavage means,
- ii) a first target nucleic acid, said first target nucleic acid
- 20 having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region, and said second region is located adjacent to and downstream from said third region;
- iii) a first oligonucleotide having a 5' and a 3' portion, said 5'
- 25 portion of said first oligonucleotide having a sequence complementary to said second region of said target nucleic acid and said 3' portion of said first oligonucleotide having a sequence complementary to said third region of said target nucleic acid;
- iv) a second oligonucleotide having a 5' portion, a central portion
- 30 and a 3' portion, said 5' portion of said second oligonucleotide having a sequence complementary to said first region of said target nucleic acid, said central portion of said second oligonucleotide having a sequence complimentary to said second region of said target nucleic acid, and said 3' portion of said second oligonucleotide having a sequence that is not base-
- 35 paired to said target nucleic acid and is selected from a set of oligonucleotides, based on a following group of sequences each having a 3' and 5' portion,
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Wherein each of 1 to 3 is a nucleotide base selected to be different from the others of 1 to 3 with the proviso that up to three nucleotide bases of each sequence can be substituted with any nucleotide base provided that:

for any pair of sequences of the set:

$M_1 \leq 16$ ,  $M_2 \leq 13$ ,  $M_3 \leq 20$ ,  $M_4 \leq 16$ , and  $M_5 \leq 19$ , where:

$M_1$  is the maximum number of matches for any alignment in which there are no internal indels;

$M_2$  is the maximum length of a block of matches for any alignment;

$M_3$  is the maximum number of matches for any alignment having a maximum score;

$M_4$  is the maximum sum of the lengths of the longest two blocks of matches for any alignment of maximum score; and

$M_5$  is the maximum sum of the lengths of all the blocks of matches

having a length of at least 3, for any alignment of maximum score;  
wherein:

the score of an alignment is determined according to the equation  $(A \times m) - (B \times mm) - (C \times (og + eg)) - (D \times eg)$ , wherein:

for each of (i) to (iv):

- (i)  $m = 6$ ,  $mm = 6$ ,  $og = 0$  and  $eg = 6$ ,
- (ii)  $m = 6$ ,  $mm = 6$ ,  $og = 5$  and  $eg = 1$ ,
- (iii)  $m = 6$ ,  $mm = 2$ ,  $og = 5$  and  $eg = 1$ , and
- (iv)  $m = 6$ ,  $mm = 6$ ,  $og = 6$  and  $eg = 0$ ,

A is the total number of matched pairs of bases in the alignment;

B is the total number of internal mismatched pairs in the alignment;

C is the total number of internal gaps in the alignment; and

D is the total number of internal indels in the alignment minus the total number of internal gaps in the alignment; and

wherein the maximum score is determined separately for each of (i), (ii), (iii) and (iv).

5

v) a second target nucleic acid, distinct from said first target nucleic acid, and having a fourth region, a fifth region and a sixth region, wherein said fourth region is located adjacent to and downstream from said fifth region, and said fifth region is located adjacent to and downstream from said sixth region, said fifth region having a sequence complementary to said 3' portion of said sequence selected from the group of sequences listed in step (a)(iv), said sixth region having a sequence complementary to said 5' portion of the sequence selected from the group of sequences in step (a)(iv);

15 vi) a third oligonucleotide having a 5' portion, a central portion and a 3' portion, said 5' portion of said third oligonucleotide having a sequence complementary to said fourth region of said second target nucleic acid, said central portion of said third oligonucleotide having a sequence complementary to said fifth region of said second target nucleic acid, and said 3' portion of said third oligonucleotide having a sequence that is not base paired to either said second target nucleic acid or said first target nucleic acid and is selected from a set of oligonucleotides based on the group of sequences listed in step (a)(iv) such that said sequence selected is distinct from said sequence selected in step (a)(iv);

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5           b)     mixing said cleavage means, said first target nucleic acid, said  
second target nucleic acid, said first, second, and third oligonucleotides to  
create a reaction mixture under reaction conditions such that at least said  
5' portion of said first oligonucleotide is annealed to said first target  
10   oligonucleotide is annealed to said first target nucleic acid so as to create  
a first cleavage structure and wherein the combined melting temperature of  
said complementary regions within said 5' and 3' portions of said first  
oligonucleotide when annealed to said first target nucleic acid is greater  
15   than the melting temperature of said 5' and central portion of said first  
oligonucleotide, wherein cleavage of said first cleavage structure occurs to  
generate a first non-target cleavage product, and wherein at least said 5'  
portion first non-target cleavage product is annealed to said second target  
nucleic acid and at least said 5' and central portion of said third  
20   oligonucleotide is annealed to said second target nucleic acid so as to  
create a second cleavage structure and wherein the combined melting  
temperature of said complementary regions within said 5' and 3' portions of  
said non-target cleavage product when annealed to said second target nucleic  
acid is greater than the melting temperature of said 5' and central portion  
25   of said third oligonucleotide, wherein cleavage of said second cleavage  
structure occurs to generate a second non-target cleavage product; and

          c)     detecting said second non-target cleavage product.

64.    The method of claim 63, wherein said first target nucleic  
acid is genomic DNA and said second target nucleic acid is synthetic  
DNA.

30       65.    The method of claim 64, wherein said synthetic DNA has at  
least one hairpin loop.

66.    The method of claim 65, wherein the method includes a plurality  
of said first target nucleic acid sequences, a plurality of first  
oligonucleotide molecules, a plurality of said second oligonucleotide  
35   molecules, a plurality of said second target nucleic acid sequences and a  
plurality of third oligonucleotide molecules.

67.    A method of analyzing a biological sample comprising a plurality of  
target nucleic acid molecules for the presence of a mutation or polymorphism  
at a locus of each target nucleic acid molecule, the method comprising:

40       a)     providing:  
          i)    a cleavage means,

- 5           ii) a first target nucleic acid, said first target nucleic acid having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region, and said second region is located adjacent to and downstream from said third region;
- 10           iii) a first oligonucleotide having a 5' and a 3' portion, said 5' portion of said first oligonucleotide having a sequence complementary to said second region of said target nucleic acid and said 3' portion of said first oligonucleotide having a sequence complementary to said third region of said target nucleic acid;
- 15           iv) a second oligonucleotide having a 5' portion, a central portion and a 3' portion, said 5' portion of said second oligonucleotide having a sequence complementary to said first region of said target nucleic acid, said central portion of said second oligonucleotide having a sequence complimentary to said second region of said target nucleic acid, and said 3' portion of said
- 20           second oligonucleotide having a sequence that is not base-paired to said target nucleic acid and is selected from a set of oligonucleotides, based on a following group of sequences each having a 3' and 5'portion,

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wherein each of 1 to 3 is a nucleotide base selected to be different from the others of 1 to 3 with the proviso that up to three nucleotide bases of each sequence can be substituted with any nucleotide base provided that:

for any pair of sequences of the set:

$M1 \leq 16$ ,  $M2 \leq 13$ ,  $M3 \leq 20$ ,  $M4 \leq 16$ , and  $M5 \leq 19$  where:

$M1$  is the maximum number of matches for any alignment in which there are no internal indels;

$M2$  is the maximum length of a block of matches for any alignment;

$M3$  is the maximum number of matches for any alignment having a maximum score;

$M4$  is the maximum sum of the lengths of the longest two blocks of matches for any alignment of maximum score; and

$M5$  is the maximum sum of the lengths of all the blocks of matches having a length of at least 3, for any alignment of maximum score;

wherein:

the score of an alignment is determined according to the equation  $(A \times m) - (B \times mm) - (C \times (og + eg)) - (D \times eg)$ , wherein:

for each of (i) to (iv):

(i)  $m = 6$ ,  $mm = 6$ ,  $og = 0$  and  $eg = 6$ ,

(ii)  $m = 6$ ,  $mm = 6$ ,  $og = 5$  and  $eg = 1$ ,

(iii)  $m = 6$ ,  $mm = 2$ ,  $og = 5$  and  $eg = 1$ , and

(iv)  $m = 6$ ,  $mm = 6$ ,  $og = 6$  and  $eg = 0$ ,

$A$  is the total number of matched pairs of bases in the alignment;

$B$  is the total number of internal mismatched pairs in the alignment;

$C$  is the total number of internal gaps in the alignment; and

$D$  is the total number of internal indels in the alignment minus the total number of internal gaps in the alignment; and

wherein the maximum score is determined separately for each of (i),  
(ii), (iii) and (iv).

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v) a second target nucleic acid, distinct from said first target nucleic acid, and having a fourth region, a fifth region and a sixth region, wherein said fourth region is located adjacent to and downstream from said fifth region, and said fifth region is located adjacent to and downstream from said sixth region, said fifth region having a sequence complementary to said 3' portion of said sequence selected from the group of sequences listed in step (a)(iv), said sixth region having a sequence complementary to said 5' portion of the sequence selected from the group of sequences in step (a)(iv);

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vi) a third oligonucleotide having a 5' portion, a central portion and a 3' portion, said 5' portion of said third oligonucleotide having a sequence complementary to said fourth region of said second target nucleic acid, said central portion of said third oligonucleotide having a sequence complementary to said fifth region of said second target nucleic acid, and said 3' portion of said third oligonucleotide having a sequence that is not base paired to either said second target nucleic acid or said first target nucleic acid and is selected from a set of oligonucleotides based on the group of sequences listed in step (a)(iv) such that said sequence selected is distinct from said sequence selected in step (a)(iv);

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b) mixing said cleavage means, said first target nucleic acid, said second target nucleic acid, said first, second, and third oligonucleotides to create a reaction mixture under reaction conditions such that at least said 5' portion of said first oligonucleotide is annealed to said first target nucleic acid and wherein at least said 5' and central portion of said second oligonucleotide is annealed to said first target nucleic acid so as to create a first cleavage structure and wherein the combined melting temperature of said complementary regions within said 5' and 3' portions of said first oligonucleotide when annealed to said first target nucleic acid is greater than the melting temperature of said 5' and central portion of said first oligonucleotide, wherein cleavage of said first cleavage structure occurs to generate a first non-target cleavage product, and wherein at least said 5' portion first non-target cleavage product is annealed to said second target nucleic acid and at least said 5' and central portion of said third oligonucleotide is annealed to said second target nucleic acid so as to

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- 5 create a second cleavage structure and wherein the combined melting  
temperature of said complementary regions within said 5' and 3' portions of  
said non-target cleavage product when annealed to said second target nucleic  
acid is greater than the melting temperature of said 5' and central portion  
of said third oligonucleotide, wherein cleavage of said second cleavage  
10 structure occurs to generate a second non-target cleavage product; and  
c) detecting said second non-target cleavage product.
68. The method of claim 67, wherein said first target nucleic  
acid is genomic DNA and said second target nucleic acid is synthetic  
DNA.
- 15 69. The method of claim 68, wherein said synthetic DNA has at  
least one hairpin loop.
70. The method of claim 69, wherein the method includes a plurality  
of said first target nucleic acid sequences, a plurality of first  
oligonucleotide molecules, a plurality of said second oligonucleotide  
20 molecules, a plurality of said second target nucleic acid sequences and a  
plurality of third oligonucleotide molecules.

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